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**Short Communication**

# Mutation Analysis in Patients with 6-Pyruvoyl-Tetrahydropterin Synthase Deficiency

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## Introduction

6-Pyruvoyl-tetrahydropterin synthase (PTPS) is the second enzyme involved in the biosynthesis of tetrahydrobiopterin ($BH_4$)[1], the obligatory cofactor for the aromatic amino acid hydroxylases[2] as well as for all types of nitric oxide synthases[3]. Defects in the PTPS are the most frequent and heterogeneous variants of $BH_4$ deficiency[4]. Three different forms of PTPS deficiency can be distinguished: (i) A central or severe type, where patients do not have sufficient PTPS activity in the brain and in the liver. Patients with this type of defect suffer from neurotransmitter deficiency in the central nervous system (CNS) and elevated phenylalanine concentrations in blood and tissue. They need to be treated with $BH_4$, L-Dopa, Carbidopa and 5-hydroxytryptophan to prevent irreversible brain damage. (ii) In the partial or peripheral type, patients do not have enough PTPS in the liver, but neurotransmitter biosynthesis in the brain is normal. They have to be treated with $BH_4$ monotherapy or follow a phenylalanine low diet[5]. (iii) A transient form, where patients present clinical symptoms only in the neonatal period, has been described in a few cases[6].

Since the human liver cDNA was isolated[7], it was possible to investigate the cDNA in primary skin fibroblasts from PTPS deficient patients in order to find out the mutations causing the disease.

In this work three new mutations, a missense mutation and two deletions, in two patients suffering from the severe type of PTPS deficiency are described. Furthermore, a summary of all mutations found so far in the cDNA of PTPS deficient patients and of the clinical data of these patients is given. We hope that it will become possible to differentiate between variants of PTPS deficiency by finding and characterizing the mutations.

## Materials and Methods

Pterins in urine were measured by HPLC, after oxidation with manganese dioxide, as described previously[8].

The PTPS assay is based on the measurement of $BH_4$ derived from dihydroneopterin triphosphate (substrate) (110 μM) in the presence of NADPH (1 mM), NADH (1 mM), DHPR (220 mU), magnesium (10 mM), sepiapterin reductase (5 mU), and Tris-HCl buffer, pH 7.4 (0.1 M)[9]. Erythrocytes (50 μl) were suspended in 50 μl of 0.2 M Tris-HCl buffer, pH 7.4 and lysed by freezing and thawing. The sample was saturated with carbon monoxide for 1～2 min under slight shaking and 50 μl of the lysate were used for the assay. The biopterin was measured fluorometrically by HPLC after oxidation with

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manganese dioxide at pH 1.0~1.5.

Primary skin fibroblasts were cultured in Dulbecco's Modified Eagle Medium (Gibco) containing 10% fetal calf serum (Gibco) and 50 units of penicillin plus 50 µg/ml streptomycin. Fibroblast lysate preparations and PTPS acivity measurements of these extracts were performed as described elsewhere[10].

Total RNA was isolated from confluent fibroblast cultures following a protocol using guanidinium-thiocyamate for cell lysis and subsequent centrifugation in cesium chloride solution[11]. With oligodeoxythymidine as a primer cDNA was synthesized from this total RNA[10]. cDNA was PCR-amplified and directly sequenced using the dideoxynucleotide chain termination method (USB sequencing kit, version 2.0) according to Thöny *et al*[10].

## Case Reports

The history of the patients JRS and UT has been reported elsewhere[10].

LL is the first child of healthy, non related parents. On the 5th day the Guthrie test was slightly elevated, and the controls on day 10 and 42 were, for plasma phenylalanine, in the high normal range. His early development was normal. At the age of 4.5 months he developed a tendency to opistotonus progressing later into frequent attacks of hypertonicity of the extremities. The serum phenylalanine rose to 2840 µmol/1, and the $BH_4$ loading test, as well as the pattern of urinary pterins confirmed the diagnosis of a defective $BH_4$, biosynthesis. The child was treated with a combination of $BH_4$, L-Dopa/Carbidopa, and 5-hydroxytryptophan[12].

SS was diagnosed as hyperphenylalaninemic at the age of four days. Plasma phenylalanine ranged between 1220 and 2400 µmol/1. At the age of 5 weeks the diagnostic test for $BH_4$ deficiency was performed; the $BH_4$ loading test was positive, neopterin was increased, and biopterin was very low. Reduced PTPS activity in the patient's erythrocytes confirmed the diagnosis. SS has been on $BH_4$ and neurotransmitter therapy since the age of ten weeks. At the age of 12 years her intelligence quotient was 54[13].

## Results and Discussion

Table 1 shows four PTPS deficient patients studied by our group (JRS, UT, LL and SS) with reduced PTPS activity in fibroblasts and erythrocytes. Three other patients described by Shintaku[14, 15] and Imamura *et al.*[15] were all of the central type with reduced or absent activity of PTPS in the erythrocytes. In one patient with the central type of PTPS deficiency, described by Ashida et al.[16], the activity of the enzyme in fibroblasts and erythrocytes was 20-fold and 5-fold lower, respectively, than that of the controls. Serum phenylalanine concentrations were elevated in all PTPS deficient patients and in

Table 1. Summary of information from patients with PTPS deficiency.

| Patient (phenotype) | Urinary pterins (mmol/mol creat) | | | Serum Phe (µmol/l) | PTPS activity | | Mutation on | Alteration on |
|---|---|---|---|---|---|---|---|---|
| | Neo | Bio | %Bio* | | Erythrocytes (µU/g Hb) | Fibroblasts (µU/mg prot.) | DNA level | protein level |
| JRS (peripheral) | 6.5 | 0.63 | 9.0 | 360 | 1.5 | 0.03 | $C_{55}$ to T<br>$\Delta G_{370}$-$G_{383}$ | R16C<br>K120 → stop |
| UT (central) | 9.1 | 0.19 | 2.1 | 1200 | 0 | ≤ 0.02 | $G_{83}$ to A | R25Q |
| LL (central) | 34.3 | 0.41 | 1.2 | 1800 | 1.3 | ≤ 0.02 | $\Delta G_{178}$-$G_{180}$<br>$\Delta T_{173}$-$G_{195}$ | ΔV57<br>K54 → stop |
| SS (central) | 30.3 | 0.13 | 0.4 | 1220 | 0.5 | ≤ 0.02 | $C_{269}$ to T | P87L |
| KH** (central) | 15.6 | 0.04 | < 0.1 | 970 | 0.1 | - | $C_{268}$ to T | P87S |
| KY** (central) | 10.4 | 0.07 | 0.7 | 1550 | 0 | - | $C_{268}$ to T | P87S |
| YI** (central) | 40.8 | 0.12 | 0.3 | 680 | 1.0 | - | $C_{268}$ to T<br>$C_{295}$ to A | P87S<br>D96N |
| XY*** (central) | - | - | 5.7# | 2840 | - | 0.43 | $A_{349}$ to G | I114V |
| Controls | 1.1-4.0 | 0.5-0.3 | 18-63 | < 120 | 11-29 | 1.9-2.6 | none | none |

#Neo/Bio

* %Bio = 100*Bio/(Neo + Bio)

** Ref. 14,15

*** Ref. 16

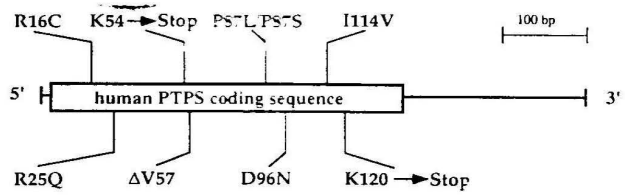


Figure 1. Human cDNA sequence encoding PTPS, and locations of mutations in patients with PTPS deficiency.

all cases initially low biopterin and high neopterin levels were detected.

Five patients were found to be homozygous for the mutations R25Q, P87L. I114V, and two patients showed the same mutation P87S (Figure 1). The other three patients harbor the compound heterozygote mutations R16C/K120 → stop, ΔV57/K54 → stop and P87S/D96N. All mutations found in the cDNA of PTPS deficient patients are in highly conserved regions. The codon at position 87 sems to be a hot spot for mutations in the 435bp reading frame of the PTPS cDNA. Four of eight analyzed patients showed a mutation at this position.

PTPS deficient patients with loower PTPS activities in erythrocytes and in fibroblasts showed also higher serum phenylalanine levels, higher neopterin and lower biopterin levels. Data presented in Table 1 and those from previous studies[17] suggested that patients with the peripheral type of PTPS deficiency presented intermediate biochemical abnormalities. Biochemical characterization including kinetic and crosslinking experiments will reveal structural and functional differences between mutant proteins and the wild type PTPS. There was so far no correlation between the phenotype and genotype.

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